

Whole-ecosystem study shows rapid fish-mercury response to changes in mercury deposition

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Edited by Deborah Swackhamer, University of Minnesota, St. Paul, MN, and accepted by the Editorial Board August 10, 2007
(received for review May 4, 2007)

Methylmercury contamination of fisheries from centuries of industrial atmospheric emissions negatively impacts humans and wildlife worldwide. The response of fish methylmercury concentrations to changes in mercury deposition has been difficult to establish because sediments/soils contain large pools of historical contamination, and many factors in addition to deposition affect fish mercury. To test directly the response of fish contamination to changing mercury deposition, we conducted a whole-ecosystem experiment, increasing the mercury load to a lake and its watershed by the addition of enriched stable mercury isotopes. The isotopes allowed us to distinguish between experimentally applied mercury and mercury already present in the ecosystem and to examine bioaccumulation of mercury deposited to different parts of the watershed. Fish methylmercury concentrations responded rapidly to changes in mercury deposition over the first 3 years of study. Essentially all of the increase in fish methylmercury concentrations came from mercury deposited directly to the lake surface. In contrast, <1% of the mercury isotope deposited to the watershed was exported to the lake. Steady state was not reached within 3 years. Lake mercury isotope concentrations were still rising in lake biota, and watershed mercury isotope exports to the lake were increasing slowly. Therefore, we predict that mercury emissions reductions will yield rapid (years) reductions in fish methylmercury concentrations and will yield concomitant reductions in risk. However, a full response will be delayed by the gradual export of mercury stored in watersheds. The rate of response will vary among lakes depending on the relative surface areas of water and watershed.

bioaccumulation | mercury methylation | stable isotopes | whole-ecosystem experimentation | methylmercury

Industrial activities have increased atmospheric mercury deposition and global mercury contamination ≈ 3 -fold since pre-industrial times (1). Some of this deposited mercury is converted by bacteria to methylmercury, a highly toxic form that bioaccumulates through food webs. In thousands of lakes in North America, Europe, and Asia, methylmercury contamination of fish negatively impacts the health of humans and wildlife, causes financial losses to commercial and sport fisheries, and affects those traditional ways of life in which fish are consumed as primary foods (2–5). This issue has now approached a critical juncture because many nations (e.g., the United States and Canada) and organizations (e.g., United Nations Environment

Program) are debating the implementation and extent of mercury emission controls. One of the reasons for this debate is that it has been notoriously difficult to establish how annual and regional patterns of mercury loading via deposition influence fish methylmercury concentrations (6). This is because the effects of mercury deposition alone are obscured by various factors [including climate change (7), lake acidification (8), and land use (9–11)] that act synergistically to influence fish methylmercury concentrations. In addition, there is reason to believe that reductions in atmospheric mercury deposition due to emission controls might not be reflected very quickly in lowered levels of methylmercury in fish: there are many decades of prior mercury deposition stored in lakes and watersheds that may continue to be methylated and bioaccumulated even after deposition rates are reduced. However, if contemporary mercury loadings are the more important for methylation, then a change in loading should be reflected in fish relatively quickly. As a result of these uncertainties, it has been impossible, until now, to predict how quickly fish mercury concentrations would change if and when atmospheric mercury loadings are reduced.

To overcome the complications described above, we combined two unique experimental approaches. First, manipulation of an entire catchment (a lake and its watershed) was used to examine the impacts of mercury loadings at the whole-ecosystem scale. This scale was necessary because mercury works its way through catchments in many steps that interact with each other in ways that are not completely understood: beginning with deposition

Author contributions: R.C.H., J.W.M.R., M.A., K.G.B., P.J.B., B.A.B., C.C.G., J.A.G., A.H., H.H., J.P.H., C.A.K., D.P.K., S.E.L., M.J.P., C.L.P., A.R., and V.L.S.L. designed research; R.C.H., J.W.M.R., M.A., C.L.B., K.G.B., P.J.B., B.A.B., C.C.G., J.A.G., A.H., H.H., J.P.H., C.A.K., D.P.K., S.E.L., R.P.M., M.J.P., C.L.P., A.R., K.A.S., G.R.S., V.L.S.L., and M.T.T. performed research; M.A., K.G.B., P.J.B., B.A.B., C.C.G., J.A.G., A.H., H.H., J.P.H., C.A.K., D.P.K., S.E.L., R.P.M., M.J.P., C.L.P., K.A.S., G.R.S., V.L.S.L., and M.T.T. analyzed data; and R.C.H., J.W.M.R., M.A., P.J.B., R.A.B., B.A.B., C.C.G., J.A.G., A.H., H.H., J.P.H., C.A.K., D.P.K., S.E.L., M.J.P., C.L.P., K.A.S., and V.L.S.L. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. D.S. is a guest editor invited by the Editorial Board.

Freely available online through the PNAS open access option.

Abbreviations: ELA, experimental lakes area; ICP, inductively coupled plasma.

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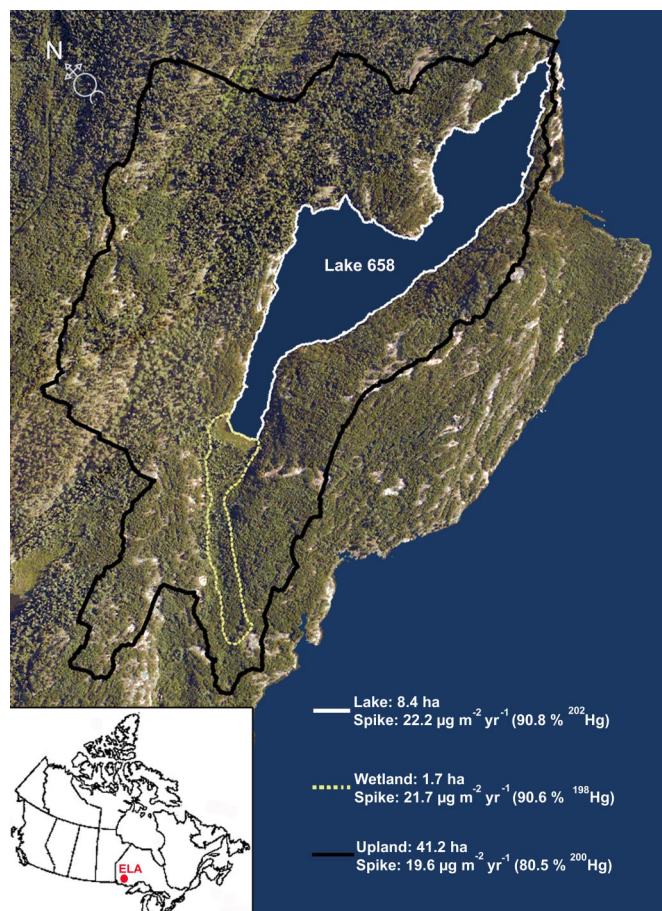


Fig. 1. Three-year average isotopic mercury addition rates (2001–2003) to the upland, wetland, and lake surfaces of the Lake 658 ecosystem at the ELA, northwestern Ontario. The target rate was $22 \mu\text{g m}^{-2} \text{year}^{-1}$. The average actual addition rate for the whole catchment was $20.1 \mu\text{g m}^{-2} \text{year}^{-1}$, which was 6 times the average wet deposition to this site ($3.2 \mu\text{g m}^{-2} \text{year}^{-1}$) over the same period.

onto vegetation and soils, mercury is mobilized into streams, methylated in a variety of different habitats within an ecosystem, and then bioaccumulates through complex food webs. Thus, the real-world predictive value of laboratory-scale and mesocosm experiments is limited. Second, the addition of enriched stable mercury isotopes enabled us to follow newly deposited mercury separately from mercury that was already in the catchment. This experiment made it possible to measure the magnitude of change in fish methylmercury concentrations due solely to changes in mercury input and to examine how quickly methylmercury concentrations in fish change in response to alterations in annual mercury loading.

The Mercury Experiment to Assess Atmospheric Loading in Canada and the United States (METAALICUS) is being conducted in the Lake 658 catchment at the Experimental Lakes Area (ELA), northwestern Ontario (Fig. 1), where wet deposition of mercury is low compared with other more contaminated sites in Europe and the United States (refs. 1 and 12 and Fig. 1). Here, inorganic mercury load to an entire catchment could be experimentally increased to levels experienced in more polluted regions (1) by adding mercury in the form of three highly enriched stable isotopes. Because the wetland, upland, and lake portions of the ecosystem were expected to respond differently to mercury loadings, study of the catchment was partitioned into these three compartments, and a different isotope was added to each one. Specifically, wetlands and lake sediments are sites of

intense mercury methylation, and uplands are important contributors of inorganic mercury to lakes (13, 14). In upland and wetland areas, isotopic mercury (referred to as upland spike and wetland spike) was added by fixed wing aircraft once each year for 3 years (2001–2003). Lake spike was added by boat every 2 weeks during the open-water season (Fig. 1). In this paper, the term “ambient mercury” refers to all mercury that is not spike mercury. In ELA catchments, most of the mercury is atmospheric in origin, with $\approx 1\%$ estimated to come from geologic weathering (15). Here, we describe results of the first 3 years of mercury additions.

Results and Discussion

After 3 years, all of the mercury spikes were still moving from their points of application, the vegetation and surface water, to soils and sediments, which are the dominant sites of long-term mercury accumulation in ecosystems (Table 1). In the upland, the areal mass of ambient mercury was much greater in the soil than in vegetation, whereas spike mercury was only slightly greater in the soil. In the wetland, ambient mercury was likewise much greater in the peat than in vegetation, whereas spike mercury had the opposite distribution, being more predominant in vegetation than in peat. In the lake, both spike and ambient mercury masses were greater in the sediments than in the water, but the contrast was much less for spike mercury (Table 1).

Throughout this article, the effects of spike additions are expressed as the percentage increase in mercury or methylmercury that resulted from the added isotopic form [(spike mercury/ambient mercury) $\times 100$]. For the large soil, peat, and sediment pools, the percentage increases over 3 years were small (2–5%; see Table 1).

Although spike export via runoff from upland and wetland to the lake was minimal, both areas yielded substantial amounts of ambient mercury (Fig. 2). In fact, the quantity of upland spike observed in runoff, although detectable, was ≈ 100 times less than the amounts of ambient mercury (Fig. 2a). This ratio in runoff was similar to the ratio of spike to ambient mercury concentrations observed in watershed soils (Table 1). The amount of wetland spike exported was below the level of detection (Fig. 2b). Thus, after 3 years of loading, most of the spike mercury remained bound to vegetation and soils, and $\approx 99\%$ of the mercury in runoff was older, preexisting ambient mercury. A similar result was seen in a pilot study on an upland microcatchment (16) in which spike mercury worked its way into the soils and was incorporated into the much larger preexisting pool of soil mercury before exiting the catchment.

Although the increase of mercury exported to Lake 658 due to spike additions to upland areas of the catchment was insignificant, it became larger each year (0.1% in 2001, 0.3% in 2002, and 0.6% in 2003) and therefore is expected to increase further with continued loading. Over the long term (centuries), historical data suggest that watershed export of mercury may respond proportionately to changes in atmospheric mercury (17, 18).

Because upland and wetland mercury exports were essentially unchanged ($<1\%$) by spike additions, the only significant increase in mercury loading to the lake occurred as a result of spike additions directly to the lake itself. In fact, lake spike constituted the single largest input of mercury to the lake (Fig. 3). The next largest input was old ambient mercury from the uplands, followed by wet deposition of ambient mercury and old ambient mercury from the wetland (Fig. 3). Ambient mercury inputs actually decreased from 2001 to 2003 due to natural variations in rainfall and stream flow that resulted in corresponding changes in ambient mercury runoff. Overall, the spike applications increased the average annual mercury load to the lake by 120%.

Essentially, all mercury in fish is methylmercury (19), produced by anaerobic bacteria acting on inorganic mercury before

For the canopy, vegetation masses exclude tree boles. The depths of soil, peat, and sediment chosen were based on the depth of penetration of spike mercury after 3 years. For upland soils, this was the entire soil column. For peat and the lake sediments, this was the upper 5 cm. The total spike amounts, per meter squared, were 68%, 74%, and 45% of the spike applications over 3 years to the upland, wetland, and lake (Fig. 1), respectively. Independent measurements found that 25–30% of upland and wetland spikes (unpublished data) and 45% of the lake spikes (37) were lost through evasion to the atmosphere. An additional 5% of lake spike was lost through the lake outflow. Thus, all of the spike applied could be accounted for within the uncertainties of the measurements. gDW, grams dry weight.

Some of the spike added directly to the lake was transported rapidly to sites of methylation in sediments and anoxic bottom waters. Methylated lake spike was found in the anoxic bottom

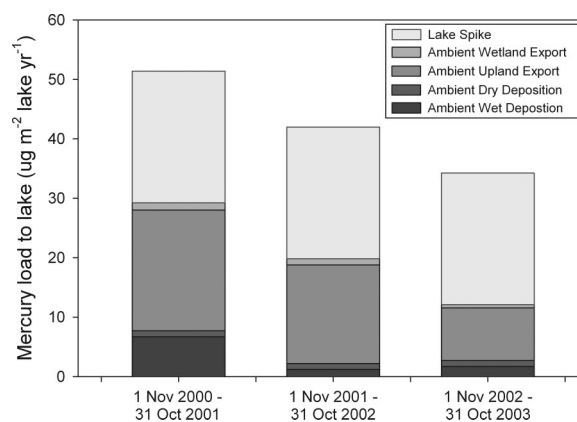


Fig. 3. Annual loadings of spike and ambient mercury to Lake 658 per square meter of lake surface.

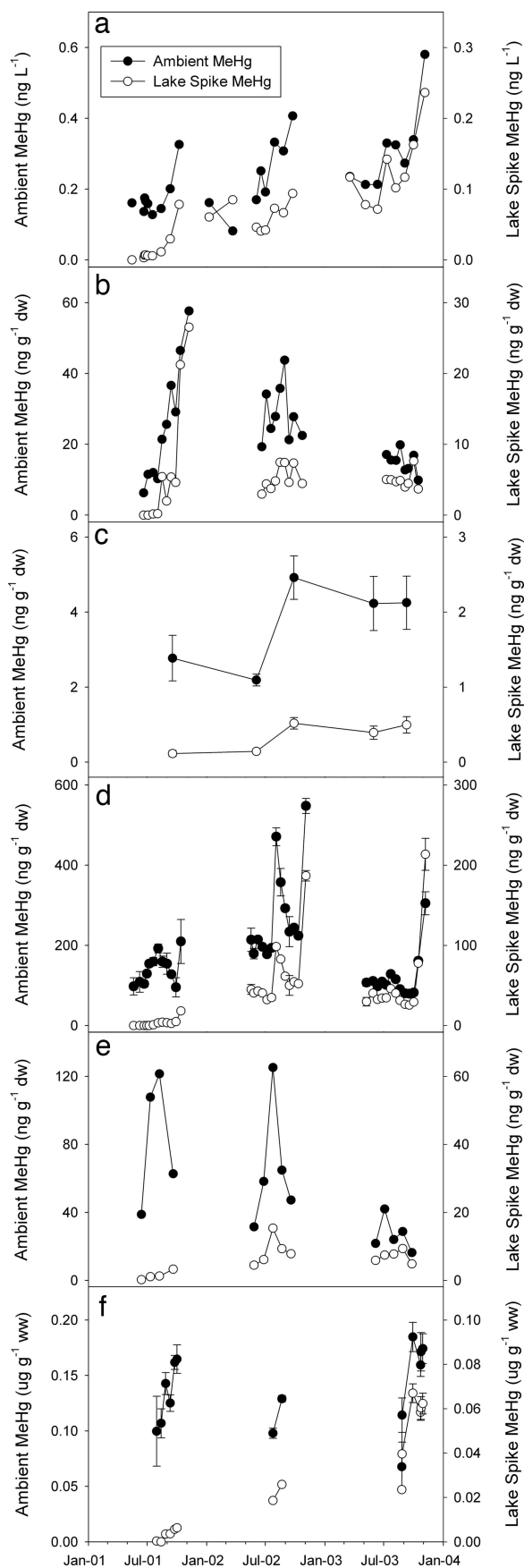


Fig. 4. Concentrations of lake spike and ambient methylmercury (MeHg) in water (a), sediment trap material (b), the top 2 cm of sediments (c), zooplankton

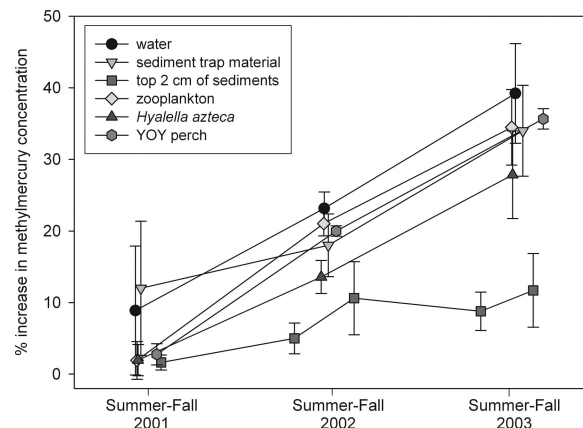


Fig. 5. Percentage increase of methylmercury concentrations in water, sediment trap material, top 2 cm of sediments, and biota resulting from additions of lake spike mercury to Lake 658 over a 3-year period. Vertical bars are standard deviations on the seasonal means. All trends were significantly linear with time ($P < 0.05$), except for the sediment traps and zooplankton.

waters (at a concentration of $0.014 \text{ ng} \cdot \text{liter}^{-1}$) 3 days after first being added to the surface water (Fig. 4a). It was found in sediment traps below the thermocline 2–4 weeks after the first lake spike additions (Fig. 4b). After 1 month, methylated lake spike was found in sediments, zooplankton, and benthos (*Hyalella azteca*) (Fig. 4c, d, and e, respectively), and within 2 months, it was found in several fish species in Lake 658 (22), including young-of-the-year yellow perch (Fig. 4f). In contrast, upland spike was not detected in benthos or fish until the third year of additions and even then was not present in quantifiable amounts. Wetland spike was never detected in the biota.

Week-to-week patterns of lake spike and ambient methylmercury concentrations in the lake water, sediments, and food web were remarkably similar (Fig. 4). In all of these compartments, whenever ambient methylmercury concentrations increased or decreased, so did the concentration of lake spike methylmercury. This synchrony means that the same environmental factors that act on the ambient mercury being methylated and bioaccumulated were also acting on the lake spike, and that bioavailable spike mercury behaved like bioavailable ambient mercury.

There was year-to-year variability in concentrations of both ambient and spike methylmercury in all lake compartments (Fig. 4). Annual variation in methylmercury concentration is driven by environmental factors [e.g., temperature, pH (8, 23)] that affect methylation rates. Although this natural variability has confounded earlier attempts to isolate the effect of mercury loading in ecosystems, the use of enriched stable isotopes allowed us to follow spike methylmercury separately from ambient methylmercury. Thus, we were able to determine the increase in methylmercury that was due solely to the lake spike additions. In all compartments of the lake, the percent increase in methylmercury due to the lake spike increased approximately linearly with time (Fig. 5). The rates of increase were very similar in the water, the sediment trap material, and the biota, including fish (Fig. 5). The fractional rate of increase was slower in sediments, likely because lake spike mercury was more diluted in the top 2 cm of sediment, which contains ≈ 20 years of mercury deposition.

(d), *H. azteca* (e), and young-of-the-year (YOY) yellow perch muscle samples (f) during 3 years of mercury spike additions to Lake 658 and its watershed. The perch data were measured as total mercury with the assumption that almost all mercury in fish muscle is methylmercury (19). Vertical bars are standard errors.

These data (Fig. 5) clearly show an annual, cumulative increase in methyl mercury bioaccumulation in response to the continuing experimental additions of inorganic mercury to the lake. By the end of the third year of additions, concentrations of methylmercury in water and biota were 30–40% higher than they would have been if the lake spike had not been added.

The contribution of lake spike to the methylmercury pools was greater each year (Fig. 5), even though the quantity of lake spike mercury added was the same each year. This could happen only if the inorganic mercury pool being methylated contained >1 year's worth of mercury inputs. This finding has implications for the response times of lakes to changes in mercury inputs.

The 30–40% increase in methylmercury in biota was much less than the 120% increase in inorganic mercury loading to the lake (Fig. 3). Previous studies have shown that, on the short term, there is a proportional response of rates of production (24, 25) and bioaccumulation (26) of new methylmercury to new additions of inorganic mercury. However, when new mercury is added to a system that already contains older inorganic mercury, both the new and older mercury contribute to methylation (although not necessarily equally), so on the short term the overall rate of mercury methylation (new + old) does not respond in direct proportion to the amount of new mercury added. This is why the increases in methylmercury in the first 3 years (30–40%) were much less than the increase in loading rate (Fig. 5).

We do not know yet whether there will be a proportional response to the continuing increased loading to the lake in the longer term, as suggested by some regional studies (6). For example, if mercury demethylation switched on at some increased concentration of sediment methylmercury, the end response would not be directly proportional to the increase in inorganic mercury loading.

The percentage increase in methylmercury has obviously not yet reached a plateau (Fig. 5), indicating that the ecosystem is not yet in steady state. This may not happen for a decade or more (27). Thus, our data show that even the most responsive compartment of the catchment (the lake) was not yet at a new steady state, and the terrestrial compartments will take substantially longer to achieve such a state. We expect that, in the long term, the full effect of the increased mercury loading will be much larger than the 30–40% increase in methylmercury in the biota that was evident after only 3 years.

Conclusions

Our experiment showed that an increase in mercury loading at rates relevant to atmospheric deposition resulted in an increase in methylmercury production and concentrations in aquatic biota in only 3 years. As emission controls are instituted and atmospheric mercury deposition decreases, there could be some hysteresis in how lakes respond to decreased mercury loading. However, this would not change the expectation that a decrease in atmospheric mercury deposition would lower fish mercury concentrations. Typically, lakes that receive all of their mercury from the atmosphere (such as perched seepage lakes) could be expected to respond in approximately a decade. For all other lakes, which receive at least some of their mercury load from the watershed, as well as a portion directly from the atmosphere, we would expect multiphased responses to a reduction in input: (i) an initial rapid decline in the mercury content of fish as a result of reduced direct deposition to the lake, followed by (ii) responses from the wetland and the upland, which will be prolonged declines (taking up to centuries) driven by reequilibration of the wetland peat and upland soils. Elucidation of this multiphased response advances our understanding of how anticipated reductions in atmospheric mercury emissions will affect fish methylmercury concentrations in lakes, predicting not only

that there will be a benefit but also the time scales over which that benefit will be achieved.

Materials and Methods

Site Description. Mercury Experiment to Assess Atmospheric Loading in Canada and the United States (METAALICUS) is being conducted at the ELA, northwestern Ontario (28), on the Lake 658 catchment. Lake 658 is a 13-m-deep, oligotrophic, headwater lake located in the ELA. The lake has an average water residence time of 5 years, pH \approx 6.5, dissolved organic carbon \approx 9 mg-liter⁻¹ and summer anoxia in the bottom 1–2 m of the hypolimnion (29). The terrestrial vegetation is a typical boreal forest with uplands mainly of jack pine, balsam fir, red maple, poplar, and paper birch growing on thin podzolic soils. The sphagnum moss-dominated wetland has an overstory of black spruce and an understory of alder and Labrador tea, leather leaf, and sweet gale.

Spike-Application Methods. The upland and wetland spikes were applied once each year by fixed wing aircraft. HgNO₃ spikes were diluted in acidified lake water (pH 4) in a fiberglass tank in the aircraft and then were sprayed onto the terrestrial surfaces by using a stainless steel boom actuated by global-positioning software. Spraying was carried out only immediately before or during a rain event and at wind speeds of <15 km-hr⁻¹ to minimize drift. Other application details, including losses of mercury to the equipment and an accounting of these losses, are described elsewhere (29). The net application rates (Fig. 1) to the upland and wetland areas were somewhat different from the target rate (22 μ g-m⁻²-year⁻¹) because of these losses. To prevent spray drift of terrestrial spikes into the lake, a buffer zone of 20 m was left around the shoreline of the lake. This area was sprayed manually with a gas-powered water pump and fire hose (29).

Lake spike additions were done by adding HgNO₃ lake spike to four 20-liter carbuoys filled with acidified lake water (pH 4), and dispensed into the propeller wash of an electric outboard motor. Additions were done at dusk to minimize photoreduction and loss of elemental mercury and were done at 2-week intervals over an 18-week period during the ice-free season.

Sampling Methods. During the open-water season, rain was collected into preacidified Teflon containers mounted in an automated precipitation sampler (30). Integrated snow samples were collected using a Teflon core tube, on the lake surface just before snowmelt. Average canopy (foliage and stems) mercury pools were estimated by using a Leaf Area Index (LAI) map of the watershed derived from LiDAR, an LAI–biomass relationship (31), and mercury concentrations at 20 upland and 3 wetland sites in August 2003. Ground vegetation mercury pools were estimated from areal biomass and average mercury concentrations of ground vegetation at each of the same 23 sites. Mercury masses in upland soils in the basin were estimated from coring surveys with 2.5-cm diameter polycarbonate coring tubes. For mercury concentrations, cores were taken from 88 randomly distributed sites in October 2003 and were sectioned into two major horizons: organic horizon and bottom mineral horizon. Average depths and bulk densities for the upland soil horizons were determined by a larger survey using 185 sites aligned along 37 transects. These data were combined to calculate areal mercury masses for the whole soil depth. In the wetland, cores were taken from 16 sites and were sectioned in 4-cm intervals for measurement of ambient and spike mercury concentrations. Average bulk density was determined at 18 sites and was used with mercury concentrations to calculate areal masses for the upper 5 cm of peat. In the lake, intact sediment cores were collected by diver, sectioned within a few hours and immediately frozen. Cores were taken from 11 sites in the fall of 2003 at water

depths of 0.2–11 m and were sectioned into 2-cm intervals. Mercury and bulk density were measured on the same sections and were used to calculate areal mercury mass for each site.

Mass flux of terrestrial isotopes to the lake was estimated from event and base-flow concentrations and estimates of terrestrial runoff. Volumetric flows were monitored continuously at four representative locations in the basin and were then scaled up to the full basin by using the U.S. Geological Survey's Precipitation-Runoff Modeling System (32).

Lake water samples were pumped through acid-washed Teflon tubing into acid-cleaned Teflon bottles by using the "clean hands-dirty hands" protocols (33). Zooplankton samples were taken and prepared for methylmercury analysis as described previously (34). *H. azteca* invertebrate samples were collected by kick-sampling and sweep-netting of epilimnetic sediments and prepared for analysis as described elsewhere (27). Yellow perch were collected using small-mesh gill nets (6–10 mm) set for short time periods (<15 min), and muscle samples were processed as described previously (27). All biota were frozen until analysis.

Analytical Methods. Total mercury (THg) in water was oxidized to inorganic Hg(II) by the addition of BrCl, reduced to Hg(0) using SnCl₂, purged onto gold traps, and analyzed after thermal desorption by inductively coupled plasma (ICP)/MS (35). For THg, fish tissue, soil, peat, and sediment samples were digested in HNO₃/H₂SO₄ and quantified by ICP/MS using either an on-line continuous flow cold vapor system (35, 36) or a system with preconcentration on gold traps (2600 total mercury analyzer; Tekran, Knoxville, TN) for vegetation and soils (35, 36). Many samples received aliquots of ²⁰¹Hg(II) as an internal standard. Methylmercury in freeze-dried zooplankton and zoobenthos was extracted with KOH/MeOH. The methylmercury in

water, sediment, soil, and peat samples was collected by distillation or extraction (35, 36). Methylmercury from all samples was ethylated by additions of NaBEt₄, and the volatile mercury species were purged and trapped. Samples were thermally desorbed and separated by gas chromatography before quantification by ICP/MS (35). THg and methylmercury analyses by ICP/MS were performed in four project laboratories. Interlaboratory calibrations were done on various sample types (35, 36), artifact formation of methylmercury during analysis was assessed, and routine analyses of appropriate certified reference materials were performed to ensure comparability of results among laboratories.

We thank M. P. Stainton and R. H. Hesslein for the preparation of mercury isotopes; M. Lyng (and the entire Experimental Lakes Area water sampling, hydrology, and water chemistry crews), T. Bell, J. DeWild, B. Dimock, M. Dobrin, K. Fetterly, J. Finch, S. Harriman, J. Januszkiewicz, M. Kullman, J. LeGal, C. Miller, B. Morris, M. Olson, N. Ogrinc, S. Olund, A. Poulain, M. Rearick, J. Reid, G. Riedel, J. Shead, L. Tate, T. Trinko, and J. Zhu for field collections, spike applications, and analyses of samples; N. Flood for editorial comments; and D. W. Schindler, J. Hill, and C. Whipple for helpful criticisms. We thank the following agencies for their support: Electric Power Research Institute, Fisheries and Oceans Canada, Natural Sciences and Engineering Research Council of Canada, the U.S. Environmental Protection Agency, the U.S. Department of Energy, the U.S. Geological Survey's Toxic Substances Hydrology Program, National Science Foundation Grant DEB 0451345 (to C.C.G.), Southern Company, Wisconsin Focus on Energy Program Project Grant no. 4900-02-03 (to J.P.H.), the U.S. Geological Survey/National Institutes for Water Resources Competitive Grants Program through the University of Wisconsin Water Resources Institute, and Environment Canada. This is contribution no. 11 to the The Mercury Experiment to Assess Atmospheric Loading in Canada and the United States Project.

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